filaments within the bundle with a high probability (24%). Although a single myosin Va is sufficient to transport cargo in vitro, intracellular cargo transport is driven by multiple motors. To understand the collective behavior of multiple motors, we have linked two myosin Va motors, with only one head of each motor labeled with either a red or green Qdot, via a third Qdot which acted as a cargo. If each motor walks on a different actin filament within the bundle, then the two motors may experience an off-axis load. The velocity and the run length of the 2-motor complex was reduced significantly from that of a single motor, suggesting that the motors interfere with each other's motion. Interestingly, the leading motor takes ~10% back steps, indicating that it experiences a resistive load from its partner. Both the run length of the complex and the step lifetimes of the motors were correlated to the inter-motor distance, with the run length decreasing and the step lifetimes increasing with greater motor separation. Our data suggest that the two motors step independently when close together. However, when far apart, tension increases in their cargo-linkage, which results in inter-motor mechanical coupling. This study will provide insight into the mechanism of how multiple motors mechanically interact to transport cargo in vivo.

3307-Pos Board B462

Stepping Mechanisms of Myosin Va on Various Actin Cytoskeleton Structures

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Myosin Va plays a central role in intracellular polarized transport through its actin-based processivity. Cargo transport by myosin Va has been intensively investigated. However, it is not known yet whether a myosin Va molecule moves over all the types of actin filaments in cells. To examine the effects of different types of actin filaments on myosin Va's processivity, we have used actin cytoskeleton structure in cells that were de-membraned by triton treatment. We then observed the movement behavior of single myosin Va molecules on these different areas of actin filaments which include stress fibers, filopodia, leading edges, and lamellipodia. The run-length, direction of step and step-size of myosin Va, at each region of cytoskeletal actin were measured. We found that myosin Va molecules moved well on stress fibers (run-length: 1.8 ± 0.35 μm), leading edge (run-length: 2.1 ± 0.43 μm), and filopodia (run-length: 1.6 ± 0.21 μm), but not at lamellipodia area (run-length: 0.54 ± 0.35 μm). The step-size of myosin Va molecule was 35.2 ± 8.2 nm at the stress fiber area, while it exhibited two peaks (Major peak: 34.6 ± 9.1nm, and Minor peak: 18.5 ± 5.5 nm) on filopodia. On the actin filaments at the leading edge, the distribution of myosin Va step-size was larger than others (35.5 ± 16.2 nm), suggesting that the myosin Va may take side-steps on actin filaments at the leading edges. We further investigate the movement and mechanism of myosin Va on a restricted subset of actin filaments.

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More than Just a Cargo Adapter: Melanophilin Prolongs Slow Processive Runs of Myosin Va

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Melanophilin (Mlph) is a cargo adapter protein that links melanosomes via Rab27a to myosin Va (myoVa) for transport along actin. In the absence of cargo, full length myoVa is in equilibrium between a folded, inactive and an extended, active conformation. This equilibrium causes a deviation from a hand-over-hand stepping pattern due to altered gating between the heads (Armstrong et al. 2012). Cargo binding has been suggested to activate myoVa for transport. Here we used single molecule TIRF assays at near physiological ionic strength (150mM KCl) to determine the effect of Mlph on myoVa processivity. In the absence of Mlph, Qdot labeled full-length myoVa moved at a median velocity of 443nm/s, and showed the altered stepping pattern previously seen at lower ionic strength. Addition of Mlph recruited 14-times more motors to move processively, consistent with a simple model of cargo activation. The myoVa-Mlph complex also showed increased run lengths, with many motors traveling to the ends of the actin filament. In the presence of Mlph, myoVa moved much more slowly (median velocity=75nm/s). The speed distribution was asymmetrical and similar to speeds of melanosome movement observed in vivo. In the presence of Mlph, myoVa showed normal gating between the heads, and hand-over-hand steps on actin typical of a fully-active motor. Based on mutagenesis of Mlph the enhanced processivity depended on a positively charged cluster of amino acids in the actin binding site of Mlph. This suggests that Mlph acts as an electrostatic tether to limit myoVa dissociation from actin, a property likely to favor the transfer of melanosomes to adjacent keratinocytes in vivo. More generally, our results suggest that adapter proteins which link motors to cargo can affect motor properties in ways favorable for their biological role.

3309-Pos Board B464

Engineering of the Myosin Viia Nucleotide-Binding Pocket to Create Selective Sensitivity to N^6-Modified ADP Analogs

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Mutations in myosin VIIa (MYO7A), an unconventional myosin, lead to sensory disorders that include congenital deafness or progressive hearing loss. MYO7A that is expressed in the cochlear neuroepithelium has been shown to play a role in maintenance of tension and the organization of stereocilia. Recent studies showed that MYO7A is located at the upper end of the tip link (Grati et al. 2011), which led us to ask whether MYO7A plays a role in adaptation, a role previously thought to be performed by myosin 1c (MYO1C). Identification of the Y61G mutation in MYO1C, which is selectively inhibited by the N^6-modified ADP analog N^6-(2-methylbutyl)ADP, motivated the development of MYO1C-Y61G transgenic mice, which were used to evaluate MYO1C's role in transduction and adaptation. We engineered and characterized an analogous MYO7A mutant, Y114G-S1-SAH, to test ADP analogs for selective inhibition of mutant MYO7A. MYO7A-Y114G-S1-SAH was created through site-directed mutagenesis, and MYO7A-S1-SAH (WT) and MYO7A-Y114G-S1-SAH were expressed in SF9 insect cells with a baculoviral expression system. An NADH-coupled steady-state assay showed that the basal ATPase of MYO7A-Y114G-S1-SAH is the same as WT ($0.16s^{-1}$); at $2.25s^{-1}$, the actin-activated ATPase activity of the mutant is about 4-fold higher than WT. The rate of ADP release from the Y114G-actomyosinADP complex is 10-fold faster ($k_{-AD}=10s^{-1}$) compared to WT. The rate of ATP hydrolysis (k_H+k_{-H}) measured by tryptophan fluorescence is $>12s^{-1}$, similar to WT. MYO7A-Y114G-S1-SAH had an affinity of 0.36 μM for the N^6-(2-methylbutyl)ADP, 71-fold higher than WT (25.6 μM). These data show that activity of Y114G-MYO7A is similar to WT, except that it can be selectively inhibited by N^6-modified ADP analogs. A mouse line is being generated with the Y114G mutation knocked into the *Myo7a* locus for electrophysiological studies of transduction and adaptation.

3310-Pos Board B465

Myosin-XXI, a Motor with many Missions

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Myosin-XXI is one of only two myosins found in the Leishmania parasite genome. While no expression of myosin-IB has been found in the organism to date, myosin-XXI has been detected in both the amastigote and promastigote stages of the Leishmania life cycle. The presence of only a single myosin isoform suggests that this myosin carries out a variety of functions within the protozoa, including membrane anchorage, longer range directed movements of cargo and possibly roles in cell signalling. Our aim is to investigate how a single myosin can carry out several different tasks within the cell and to identify molecular mechanisms controlling this. To determine the directionality of motor movement we performed gliding filament assays using myosin-XXI constructs expressed using a baculovirus/SF21 system and dual labelled F-actin with actin-filaments capped by gelsolin and labelled with phalloidin-TRITC at their barbed ends and phalloidin-FITC at their pointed ends. These experiments showed that myosin-XXI is a plus-end directed motor. Our in vitro studies also showed that myosin-XXI binds to a variety of lipids including PIP2 and PIP3 as well as a number of other phospholipids. Furthermore, the motor can adopt both a monomeric and a dimeric conformation in vitro. using a variety of tail constructs we found that only the monomeric conformation has the ability to bind lipids. We identified several distinct lipid-binding sites in the tail domain with different lipid binding specificities. Preliminary data suggest that motor dimerisation and lipid binding are regulated by binding of calcium-calmodulin which might play a key role in the cellular distribution of the motor and its ability to perform a variety of motile roles within the parasite. Sponsored by DFG-SFB 863 and Baur-Stiftung.

Actin and Actin-binding Proteins

3311-Pos Board B466

Structural Polymorphism of F-Actin is Coupled with its Mechanical Properties

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Actin plays a major role in many cellular processes including cell motility, cell division, endocytosis, and exocytosis. External and internal mechanical forces lead to cytoskeletal rearrangements in the cell. Recently, we proposed that actin filaments may be directly involved in mechanosensing. Single molecule experiments suggest that F-actin is stiff with respect to extension. We used cryo-electron microscopy and image analysis to examine variation in the axial rise (the distance between two adjacent actin protomers along the one-start left handed helix) within frozen-hydrated actin filaments. We show that F-actin can be found in both stretched and compressed structural states. The magnitude of such mechanical deformation is far beyond what has been suggested for F-actin, and the axial rise can vary from 25 to 30 Angstroms. We demonstrate that the structural state of the filament strongly correlates with the variability of the axial rise, and actin's stretching stiffness depends upon the structural state of the subdomain 2, just as we had previously shown for the bending stiffness. We show that actin binding proteins that cross-link adjacent actin protomers, such as cofilin and the actin binding domain 2 of fimbrin, dramatically reduce the variability of the axial rise within the decorated filaments. Our results demonstrate the coupling between the mechanical properties of F-actin and its structural state and provide a structural basis for actin's role in mechanosensing.

3312-Pos Board B467

Cyclic Mechanical Reinforcement of Actin Interactions

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The fundamental mechanism underlying force regulation in actin dynamics is not understood. To investigate actin depolymerization under dynamic force environments, single molecule experiments were conducted using atomic force microscopy (AFM).

A custom-made AFM and force-clamped experimental procedures were used. To measure G-actin/G-actin (GG) interaction, G-actin was immobilized on the AFM cantilever tip and the polystyrene petri-dish was functionalized with G-actin. For G-actin/F-actin (GF) interaction, F-actin was prepared and immobilized on the petri-dish. To mimic dynamic force, once binding was detected during the tip retraction, force was applied to it via one of the programming paths; (1) loading the bond to 10, 15, 20 and 25 pN forces, and then reducing the force to 5 pN; (2) loading 1.5-, 2.5- or 3.5-cycle with a 10 pN peak force and holding at 10 pN to measure bond lifetime.

GG and GF dissociations were qualitatively similar to each other. When loaded by a linear ramp, actin dissociations exhibited a biphasic transition from a catch bond to a slip bond. In the catch bond region, bond lifetimes increased as force increased to a maximum at 12 pN in GG and at 20 pN in GF interactions. Interestingly, after applying cyclic forces, the post-priming bond lifetimes of GG and GF were significantly prolonged at low force range (5-10 pN), instead of reverting instantly to a low affinity state. This shows that cyclic force is more effective in strengthening actin-actin bonds than a linear ramp.

Our study demonstrated that kinetics of actin depolymerization is force dependent and the mechanical priming process by cyclic force application significantly enhanced bond lifetime of actin/actin. We hypothesize that the mechanical reinforcement of actin/actin interaction is an important regulatory mechanism underlying cytoskeletal dynamic rearrangement by affecting the actin depolymerization kinetics.

3313-Pos Board B468

Toxoplasma Gondii Actin Assembles via Isodesmic Polymerization

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The protozoan parasite Toxoplasma gondii relies on actin polymerization for motility and host cell invasion. Despite this strict requirement for actin filaments, T. gondii actin (TgACTI) remains largely unpolymerized in vivo and recombinant TgACTI only forms short filaments in vitro. TgACTI has several amino acid substitutions when compared with muscle actin, and our previous work showed that these changes significantly diminish the lateral contacts and interactions within the actin filament. The net result is that the two strands of the filament assemble with less cooperativity and not surprisingly this has significant effects on the polymerization kinetics. Here we show that the highly unusual characteristics of TgACTI result from isodesmic polymerization rather than the nucleation-elongation kinetics of conventional eukaryotic actins. TgACTI polymerization kinetics lack a lag phase and critical concentration, and all of the results from dynamic light scattering, dilution induced depolymerization, and sedimentation can be fit using a single pair of kinetic rate constants. These findings expand the repertoire of how actin functions in cell motility and offer clues about the evolution of self-assembling, stabilized protein polymers.

3314-Pos Board B469

Interplay of Stochastic Processes during Actin Depolymerization

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The dynamic polymerization (assembly) and depolymerization (disassembly) of actin filaments are pivotal for cell motility, cell adhesion, and cell division. The hydrolysis of actin-bound ATP destabilizes the filaments over time by enhancing the depolymerization velocity. However, abrupt changes of depolymerization were observed in single filament experiments, which were interpreted in terms of global structural transitions leading to increased filament stability with aging [Kueh et al. (2009), Science 325: 960]. In contrast to this proposal, we have demonstrated that these interruptions are caused by local transitions which turned out to be the photo-induced dimerization of neighboring filament subunits [Niedermayer et al. (2012), PNAS 109: 10769]. Here, we discuss and generalize the idea that led to this crucial notion of local transitions within the filaments. The time from the initiation of depolymerization until the occurrence of the first interruption represents a stochastic variable and the distribution function of this variable is a fingerprint of the unknown transition mechanism. By modeling the underlying stochastic processes - association and dissociation of actin subunits as well as hypothetical transitions -, we compute the distribution functions for many possible transition mechanisms and compare these functions to experimental data. We also generalize our theoretical description to accommodate for instance finite size effects or the influence of ATP hydrolysis. Furthermore, we outline how stochastic modeling may help deciphering other fundamental issues in actin dynamics.

3315-Pos Board B470

Actin Filament Nucleation by Smooth Muscle Leiomodin-1

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The formation of new filaments is an essential, but kinetically unfavorable, step in actin cytoskeleton remodeling. In cells nucleation has to be catalyzed by proteins called nucleators that bring together at least two actin monomers and stabilize a polymerization nucleus. Among known filament nucleators Leiomodin (Lmod) is the only one specifically expressed in muscle cells. Lmod is related to the F-actin pointed-end capping protein tropomodulin (Tmod), with which it shares two actin-binding sites: a flexible N-terminal region and a leucine-rich repeat (LRR) domain. In addition, Lmod contains a unique C-terminal extension that features a WH2 domain, another actin-binding site.

There are three muscle-type specific isoforms of Lmod: smooth muscle Lmod1, cardiac and striated muscle Lmod2, and the fetal isoform Lmod3. To date, only Lmod2 has been shown to nucleate actin filaments. Lmod1 is quite different in sequence from Lmod2 (37% identity, most of which is concentrated in the LRR). Here we demonstrate that despite these differences smooth muscle Lmod1 is also an actin filament nucleator. However its activity and mechanism of nucleation vary significantly from that of Lmod2.

3316-Pos Board B471

A Novel Mechanism of Actin Nucleation by Rickettsia Sca2

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Pathogens destabilize the host cytoskeleton for their survival and for motility. Pathogen interaction with host gives an advantage of illustrating the key cytoskeleton functions like phagocytosis, micropinocytosis, autophagy, motility, membrane trafficking and signal transduction. Sca2 is a Rickettsial outer membrane protein is known to nucleate host actin and forms actin comet tails for the pathogen motility. Yet, the mechanism remains a mystery. Sca2 passenger domain is 1482 amino acid length protein and it has three WH2 domains flanked with two proline rich domains. Here, we have used a battery of biophysical approaches such as, Fluorescence, TIRF microscopy, ITC, X-ray crystallography and SAXS to determine the structural basis for nucleation and elongation of actin by Sca2. using biochemical and TIRF assays we have found that Sca2 consists of two domains, where one domain nucleates actin and the other is needed for processivity. We have determined the